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Antibacterial and Antifungal Substances from Vermont Plants

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IN THE PAST few years several workers have been interested in isolating from green plants substances that are toxic principally to bacteria (*1-5*), (*7-10, 13*)[2]. Few crude juices have been tested for toxicity to fungi although this work is proceeding slowly (*6*), (*11*). In this study an effort was made to test toxicity of some green plant juices to some bacteria and fungi. Several crude juices showed antagonistic action and many showed stimulation. Particular attention was directed toward those showing fungicidal or fungistatic properties.

Methods

Because the object was to test antibacterial and antifungal activity, Robbins' media (*12*), which support both organisms, were used in screening tests. However, bacteria did not grow exceptionally well on these media, although the fungi produced excellent growth. The medium for bacterial growth was changed after screening tests were completed. Media were adjusted to *p*H 7 by twentieth normal sodium hydroxide.

Since toxic substances in crude juices were thought to be in low concentration, a new reservoir other than the customary pennicylinders was sought. Absorbent cotton rolls served the purpose of holding 1-1.5 ml. of crude extract. Rolls were made by slicing No. 2 dental cotton rolls into short cylinders 1 cm. in length. Sterile cotton rolls were held by straight nose forceps, dipped into the extract, and carefully placed on agar plates. There was no runoff from the rolls because the excess was withdrawn when the forceps were released. Rolls also adhered firmly to the upper agar layer. More careful tests were run using pennicylinders. Following popular technique, petri dishes were filled with 20 ml. of Robbins' agar on a level table, allowed to harden for one hour, then incubated at 40°C. While still warm, plates were flooded by pipetting 3 ml. of agar containing suspended cells of bacteria or fungi. Reservoirs were placed immediately after the flood agar had hardened. Bacterial suspensions were made by adding 10 ml. of broth suspension to 100 ml. of 2 percent agar held at 37°C. in a

[1] Plant pathologist, biochemist, and assistant chemist, respectively.
[2] Italic figures in parentheses refer to Literature Cited.

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water bath. Fungus spore agar suspensions were made by washing spores from agar slants with sterile distilled water, adding 10 ml. suspension to 100 ml. of 2 percent agar.

Organisms used for preliminary screening were *Staphylococcus aureus* A.T.C.C. 6538P, *Escherichia coli* A.T.C.C. 9637, *Monilinia* (*Sclerotinia*) *fructicola* A.T.C.C. 9962, *Colletotrichum lindemuthianum* Vt.-2 (laboratory isolate), and *Rhodotorula glutinis,* Vt.-3. *Monilinia* and *Colletotrichum* were chosen as representative plant pathogens.

Water and alcohol extracts were prepared from plant parts after they were dried at 85°F. and ground to a fine powder. The extracts were prepared in the following manner:

Alcohol extraction: 25 g. of dried, ground plant material were stirred for ½ hour with 100 ml. of absolute alcohol and filtered. The filtrate was concentrated in vacuo to 20 ml. and 100 ml. of water added. This solution was concentrated to 75 ml., neutralized, and sterilized by passage through a sterile Zeitz filter. One milliliter is equivalent to 0.33 g. of dry material.

Water extraction: 50 g. of dried, ground material were stirred for ½ hour with 500 ml. of water in a Waring Blendor. The slurry was filtered and 250 ml. of the filtrate were concentrated to 75 ml. in vacuo, neutralized, and sterilized by passage through a sterile Zeitz filter. One milliliter is equivalent to 0.33 g. of dry material.

Tests

The following plants were tested for stimulatory and inhibitory activity toward the organisms listed above:

Ranunculaceae—*Clematis virginiana* L. (virgin's bower)
Poaceae—*Zea mays* L. (corn)
Onagraceae—*Epilobium angustifolium* L. (fireweed)
Chenopodiaceae—*Chenopodium album* L. (pigweed)
Tropaeolaceae—*Tropaeolum majus* L. (nasturtium)
Cucurbitaceae—*Cucumis melo* L. (muskmelon)
Rosaceae—*Spiraea tomentosa* L. (hardhack)
Compositae—*Cichorium intybus* L. (common chicory)
Balsaminaceae—*Impatiens biflora* Walt. (touch-me-not)
Solanaceae—*Lycopersicon esculentum* L. (tomato)
Portulacaceae—*Portulaca oleracea* L. (common purslane)

All types of responses were observed in tests against the various organisms. There were intensities or degrees of stimulation and inhibitions in reactions to organisms, dependent upon whether alcohol or water extracts were used.

Cotton rolls measuring 10 mm. in diameter were used as reservoirs. When tested all extracts were in water. The data of this comparative qualitative test are presented in the following order: test organism; plant source; nature of extraction—water (W) or alcohol (A); inhibition zone in millimeters.

Monilinia (*Sclerotinia*) *fructicola* was stimulated by extracts from *Zea* (W) and (A), and *Lycopersicon* (W), and inhibited by *Clematis* (A) 29, *Zea* G134[3] (A) 18, *Zea* GB797[3] (A) 28 and (W) 25, *Zea* No. 4528[3] (A) 27, *Chenopodium* (A) 18, *Tropaeolum* (A) 35 and (W) 30, *Cucumis* (A) 55, *Cichorium* (A) 19, and *Impatiens* (A) 30 and (W) 17.

Colletotrichum lindemuthianum, the organism causing bean anthracnose, was stimulated by *Lycopersicon* (A). Many extracts produced only slight zones of inhibition. Where germination and growth were affected, it was marked—*Cucumis* (A) 50, *Impatiens* (A) 60.

The yeast *Rhodotorula glutinis* was an excellent test organism. No stimulations were observed. However, pronounced zones of inhibition were produced by *Zea* GB797[3] (W) 12, *Zea* No. 4473[3] (W) 30, *Zea* No. 4528[3] (W) 12, *Tropaeolum* (W) 12, *Cichorium* (W) 15, *Impatiens* (A) 40, and *Lycopersicon* (W) 12.

Practically all extracts were inactive against *Staphylococcus aureus*. No stimulation was observed. Inhibition was produced by *Portulaca* (A) 21.

Escherichia coli A.T.C.C. 9637 was stimulated by *Zea* GB797[3] (A) and (W), *Tropaeolum* (W), *Spiraea* (A) and (W), and *Lycopersicon* (W); and inhibited by *Portulaca* (W) 17, and *Epilobium* (A) 16 and (W) 16.

Discussion

It is interesting to note that the majority of extracts tested were active against *Monilinia*. The yeast *Rhodotorula* was also sensitive to several extracts and reacted to all the extracts that produced inhibitory zones against *Monilinia*. *Colletotrichum* is a good manipulative organism, but it evidently is not as sensitive to such crude juice extracts, judging from the results obtained. The bacteria were not outstandingly sensitive to any of the extracts. The media used might explain some of the results observed, or the bacteria may be generally more resistant to such substances.

The three most active extracts against the fungi were *Impatiens*, *Cucumis*, and *Tropaeolum*. Work was repeated on all these extracts. It appeared that the *Impatiens* extract was very active, producing a very clear well-defined zone. *Cucumis* was active, but if plates

[3] Iowa State corn varieties.

were allowed to remain, the fungi grew back into the inhibitory zone area. Difficulty was encountered with the *Tropaeolum* (nasturtium) extracts. The extracts made from the first dried samples were active in repeated tests, but new plant material was impotent. It has not been possible to solve this problem. The first *Tropaeolum* plants were gathered outside; the second were grown in the greenhouse. It was impossible to obtain seed similar to the first nasturtium collected. It is also possible that color of flower may have influenced results. The first plants were mixed orange and yellow and the second sample was a yellow variety.

Because the *Impatiens* extracts appeared to be the most active, they were selected for further tests. Work has progressed on the isolation and identification of the fungicidal material present and will be reported elsewhere.

Summary

In this investigation 73 plant extracts were tested for antagonistic activity. The most active reactions are listed.

Several antifungal and a few antibacterial reactions were observed. The most active plant extract was that obtained from *Impatiens biflora* (wild touch-me-not). This extract was toxic to two plant pathogens and a yeast.

A new technique in screening such plant materials is given.

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